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Anti-bacterial properties of olfactory ensheathing cells and the primary olfactory pathway

by
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Statements

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Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Name and School = **Julie A Harris, School of Medicine**
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Author details and their roles:

Paper 1, : Olfactory ensheathing cells: nitric oxide production and innate immunity
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Glia 57:1848-1857, 2009
Located in chapters 3 and 4
Candidate conducted the experiments, with authors West and Chuah contributing to the idea and design of the project.

Paper 2, : *Cytokines and olfactory bulb microglia in response to bacterial challenge in the compromised primary olfactory pathway*
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J Neuroinflammation 9:109, 2012 [DOI10.1186/1742-2094-9-109]
Located in chapter 5
Candidate conducted some of the experiments (20%), with authors West and Chuah contributing to the idea and design of the project. Other experiments and contribution by the rest of the co-authors were not undertaken as part of this thesis.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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ABSTRACT

The olfactory pathway represents a potential route for pathogens to access the central nervous system (CNS) from the nasal cavity. Since infection by this route remains relatively uncommon, powerful endogenous mechanisms for preventing microbial infection must exist, but these remain poorly understood. Olfactory ensheathing cells (OECs), glial cells which ensheath the olfactory nerves from the nasal cavity to the olfactory bulb are in a prime position to assist with host immunity. Previous studies unexpectedly revealed that OECs expressed genes associated with the immune system and were able to phagocytose bacteria. OECs may play a role in host immunity, including the production of nitric oxide (NO), a potent antibacterial and antiviral agent. In this study I show that OECs are able to detect, and respond to bacterial challenge via the synthesis of NO. OECs were incubated with *Escherichia coli* and *Staphylococcus aureus*. Processes involved in NO and nitrite (a metabolite of NO) production were analysed using immunocytochemistry, live cell imaging and high performance liquid chromatography.

The results showed that in bacteria-treated OECs nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), was detectable in the majority of OECs at between one and six hours following bacterial incubation. Three isoforms of nitric oxide synthase convert L-arginine to L-citrulline and NO. mRNA expression for inducible nitric oxide synthase (iNOS) but not for neuronal nitric oxide synthase or endothelial nitric oxide synthase, was up-regulated in bacteria-treated OECs. Expression of iNOS protein and the production of NO was higher in bacteria-incubated OECs compared to untreated OECs. In the presence of NO inhibitor NG-Methyl-L-arginine which competitively inhibits the conversion of L-arginine to L-citrulline, levels of NO and nitrite were significantly attenuated. An *in vivo* rat model was established to investigate iNOS expression in the compromised olfactory pathway. Preliminary observations following instillation of

fluorescently-labelled *S. aureus* into the damaged rat olfactory epithelium, showed the presence of iNOS expressing OECs and other iNOS expressing cells, presumably macrophages. These iNOS expressing cells were not apparent in untreated control rats.

To investigate the contribution of CX3CR1 signalling to innate immunity in olfactory tissues, I utilised the CX3CR1$^{GFP/GFP}$ mice that had enhanced green fluorescent protein (eGFP) inserted into the coding region of the CX3CR1 receptor via targeted deletion, critical for binding of its ligand, CX3CL1. As a result, microglia lacked CX3CR1 and expressed green fluorescent protein which facilitated easy visualisation of their location.

The results showed that compared to wild type mice following the instillation of fluorescently-labelled *S. aureus* into the compromised nasal cavity of CX3CR1$^{GFP/GFP}$ mice greater numbers of bacteria were observed in the olfactory bulb, many of which infiltrated the granule layer of the olfactory bulb. In the CX3CR1$^{GFP/GFP}$ mice the number of microglia in the granule layer was significantly higher rather than that in the wild type mice. However, following exposure to *S. aureus* the number of microglia in the granule layer of CX3CR1$^{GFP/GFP}$ mice showed a significant decrease, which was not observed in the wild type mice. Additionally, in CX3CR1$^{GFP/GFP}$ mice elevated numbers of iNOS-expressing cells were reduced following *S. aureus* exposure in the nasal septum and olfactory bulb that were possibly OECs, suggesting cell death. In contrast to wild-type mice, there were no changes in tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-1beta (IL-1β) expression following *S. aureus* exposure in the nasal septum and olfactory bulbs of the CX3CR1$^{GFP/GFP}$ mice.

Increased understanding of the immune response of the olfactory pathway overall, as indicated by these studies showing that CX3CR1/CX3CL1 signalling plays a key role in the immune response to bacterial challenge will be beneficial, considering that the olfactory pathway is being investigated as a potential route for drug delivery to the brain.

This thesis supports the hypothesis that OECs and their signalling to macrophages and microglia are essential components of the innate immune response against bacterial invasion of the CNS via olfactory nerves.

Publications arising out of this thesis

Harris J A, West A K, Chuah M I. 2009. Olfactory ensheathing cells: nitric oxide production and innate immunity. *Glia*. Dec; 57(16):1848-57.

Herbert R P, Harris J A, Chong K P, Chapman J, West A K, Chuah M I. 2012. Cytokines and olfactory bulb microglia in response to bacterial challenge in the compromised primary olfactory pathway. *Journal of Neuroinflammation* 9: 109.

Publications related to this thesis

Vincent A J, Choi-Lundberg D L, Harris J A, West A K, Chuah M I. 2007. Bacteria and PAMPS activate NF kappa β and Gro production in a subset of olfactory ensheathing cells and astrocytes but not in Schwann cells. *Glia.* 55:905-16.

Leung J Y, Chapman J A, Harris J A, Hale D, Chung R S, West A K, Chuah M I. 2008. Olfactory ensheathing cells are attracted to, and can endocytose bacteria. *Cell Mol Life Sci*. 65:2732-9.

Abstracts

Harris J A, Vincent A J, Chuah M I and West A K. Pathogens induce nitric oxide production by olfactory ensheathing cells. July 2007. IBRO international conference. Melbourne.

Harris J A, Ruitenberg M J, West A K and Chuah M I. Inducible production of nitric oxide by olfactory ensheathing cells in response to bacteria. January 2008 - ANS 28th annual meeting. Hobart. Tasmania.

Olfactory ensheathing cells are attracted to, and are capable of phagocytosing bacteria. Leung J Y K, Chapman J, Harris J A, Chung R S, West A K and Chuah M I. January 2008 - ANS 28th annual meeting. Hobart. Tasmania.

Harris J A, West A K, Ruitenberg M J and Chuah M I.Inducible nitric oxide synthase in response to bacterial challenge in nasal tissues of wild type, CX3CR1$^{+/GFP}$ and CX3 CR1$^{GFP/GFP}$ mice. October 2009 TLROZ2009 conference. Surfers' Paradise, Queensland.

Harris J A, West A K, Ruitenberg M J and Chuah M I. Inducible nitric oxide synthase expression in the primary olfactory pathway of wild type CX3CR1 $^{+/GFP}$ and CX3CR1 $^{GFP/GFP}$ mice following damage and bacterial challenge. January 2010 ANS 2010 conference. Sydney.

Harris, J A West, A K, Chuah, MI. Induced production of nitric oxide by olfactory ensheathing cells in response to bacteria. Invited talk. September 2008, Infectious Diseases of the Nervous System- Pathogenesis and World-wide Impact conference. Institut Pasteur, Paris.

Harris J A, West A K, Ruitenberg M J and Chuah M I. Immune responses in the compromised olfactory pathway of CX3CR1 $^{+/GFP}$ and CX3CR1 $^{GFP/GFP}$ mice. September 2010. European Macrophage and Dendritic Society Meeting 2010. Edinburgh UK.

Harris, J A West, A K, Chuah, MI. Olfactory ensheathing cells, innate immunity and nitric oxide. Invited seminar September 2010, Institute of Neurology, University College, London. UK.

Harris J A, West A K, Ruitenberg M J and Chuah M I. Immune responses to bacterial challenge in olfactory tissues of CX3CR1 $^{+/GFP}$ and CX3CR1 $^{GFP/GFP}$ mice. October, 2010, Asia-Pacific Society for Neuroscience 2010, Phuket. Thailand.

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